GOLDEN NEMATODE HANDBOOK

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
PLANT PEST CONTROL BRANCH
GOLDEN NEMATODE CONTROL PROJECT

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HICKSVILLE, L.I. N.Y. JULY 1954

FOREWORD

This handbook has been prepared to meet the needs of supervisors, crew leaders, field men and others interested in the protection of crops from nematode outbreaks. The procedures set forth in this handbook have been used on this project and found to be satisfactory, however, they are not necessarily the only methods. Through the efforts of our field workers, these methods and techniques are constantly being improved.

The text of an earlier edition, a mimeographed manual, has been revised to take into account the latest developments and procedures. In addition, a complete series of how-to-do-it photographs have been inserted.

A number of persons have aided in the preparation of this handbook, and their assistance and advice is gratefully acknowledged. William F. Mai, Department of Plant Pathology, Cornell University, L. E. Butler and V. A. Lafleur, Project personnel, and the staff of the Nematology Section, Horticultural Crops Research Branch, gave valuable advice and help on a number of technical matters. Gerald Thorne, Nematology Section, prepared the chapter on Free-Living Nematodes. Daniel O. Betz, Laboratory Supervisor, Golden Nematode Control Project, was responsible for preparation of the Laboratory Section of the handbook. Printing and lettering by Michael Mohr; photographs by Martin J. Merta, Project personnel.

JOSEPH F. SPEARS
PROJECT LEADER

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ILLUSTRATIONS

INTRODUCTION

Of the many plant pests of foreign origin that have become established in the United States, the golden nematode Heterodera rostochiensis Wollenweber, is significant since it is considered to be potentially more dangerous than any of the insects and diseases affecting the potato industry. This plant parasite has perplexed research workers, pest control officials, and growers because it is so difficult to combat. There are no symptoms that will betray its presence until it has gained a foothold in the field. Once it is established, the growing of potatoes and tomatoes is impractical except in long crop rotations.

Potatoes and tomatoes are the only crops of economic importance that are known to be attacked by this pest. Injury caused by the golden nematode is wholly to the root system, which results in delayed plant emergence, stunting and early dying of the plants, and reduction in the size and yield of the crop. From 30 to 70% reduction in potato yields has been reported from some of the heavier infested fields on Long Island.

The first record of the golden nematode was from Germany in 1881, and at that time it was considered a strain of H. schachtii. In 1909 it was established that potatoes were hosts of this nematode, but not until 1923 was it described as a completely different species by Wollenweber. This nematode attracted little attention until 1913, when it was found in Scotland. It has since been found in the European Countries of England, Ireland, Sweden, Denmark, The Netherlands, Spain, France, Finland, Belgium, and also in Peru, South America. It is now recognized as a major potato pest in Europe, especially England, where it is prevalent in 65 to 75% of the fields in the important potato-growing areas.

The golden nematode was first discovered in North America in 1941, when it was found to be responsible for damage in a field of potatoes south of Hicksville, Nassau County, Long Island, New York. The exact source and time of introduction of the pest to this country has not been determined. Plant material imported from Europe has been required to be free from soil since June 1, 1919. However, in recent years golden nematode cysts have been recovered and identified from numerous interceptions made by plant quarantine inspectors of soil particles and debris contained in plant material, bulbs, packing material, and burlap bags originating in various European Countries.

Eggs and larvae of the golden nematode pass the winter in the body of the dead female, which has become a brown thick-walled protective cyst. In the spring, if these cysts are in the presence of host-plant roots, and with favorable temperatures, the eggs hatch and the larvae migrate from the cysts to attack host-plant roots. The larvae penetrate the roots, as well as the tubers, to a point where the head is near the vascular system. After penetrating the root, the organism undergoes a series of changes, the males remaining free-moving forms and the females becoming more or less stationary. The mature males die without changing

form. The females undergo a series of growth changes with the posterior portion of the body enlarging and breaking through the outer layer of the root. The body continues to enlarge and becomes spheroid and a protective cuticle begins to form.

As the cuticle ages the cyst changes color from white to yellow or golden brown, hence the common name "golden nematode." The cyst is about the size of a small pin head and, if viable, may contain several hundred eggs. In the late stage of formation, the cysts are easily detached from the roots. Although larvae invade the root system of the host plant throughout the growing season, there is no evidence that these larvae are second generation specimens. These may be from old cysts, since it is known that not all eggs from a single cyst hatch simultaneously. Eggs may remain viable for more than 17 years.

Automobility of the golden nematode larvae is very limited, and spread is primarily caused by other factors such as: (1) soil from infested fields transported manually or by wind or water; (2) seed potatoes produced on infested soil; (3) plants from infested fields; (4) equipment, vehicles, and utensils used in infested fields; and (5) bags and other containers utilized in the harvesting, storage, or shipment of potatoes grown on infested lands.

The undramatic nature of the pest in its early stages is an aid to its survival. It is very important that the organism be recognized before crop damage becomes apparent; therefore, it is necessary that a constant alert be maintained for its presence throughout the potato and tomato-producing areas of the country. Small or isolated infestations may exist unnoticed, since plant symptoms are not reliable in the detection of the golden nematode. In the early stages build-up of populations is slow, and crop damage may not be noticeable for several years.

The most reliable method for the detection of the golden nematode is the collection of soil samples and examination of the wash residue of such samples under the microscope. By field survey procedures outlined in this manual the presence of cysts can be detected before they reach a level that will cause crop losses. From records of more than one hundred fields on Long Island surveyed by this Project, it was determined that golden nematode cysts are found for the first time when the cyst concentration is approximately one million per acre. Assuming ten viable cysts were initially introduced into a field and there was a tenfold increase per year, it would take approximately six years for a cyst population to build up to the discovery level. Crop damage may not become apparent for another three years, at which time the cyst population may be built up to one billion per acre.

From this evidence it can easily be understood that a large number of cysts may exist in a potato field in which no symptoms are noticeable on the tops or the tubers. For this reason nematodes may be found in a field that has been surveyed with negative results for several years. This is also the reason that surveys must be conducted continuously in a systematic manner.

SURVEY METHODS

THE SURVEY CREW

Personnel assigned to soil surveys normally consist of crews of two or three men, one of whom is designated as the crew leader. The number of men assigned to survey a given property or area will vary with the district in which they operate and the scope of such operations. One man can survey about 25 acres per day, therefore, the crew may be varied from one to several men, depending upon the size of fields to be surveyed.

EQUIPMENT

Each crew is provided with the following equipment: (Fig. 1). A map of the area to be surveyed; a supply of No. 12 wet-strength, double-thickness paper bags; black, wax marking crayon of good quality; a pointing trowel for each member of the crew; tape or wire staplers for sealing the samples; a bristle brush for cleaning shoes and equipment; and forms on which to record operations. Each crew leader keeps a daily log of his operations in a notebook, and at the conclusion of the survey season, these notebooks are returned to the headquarter's office.

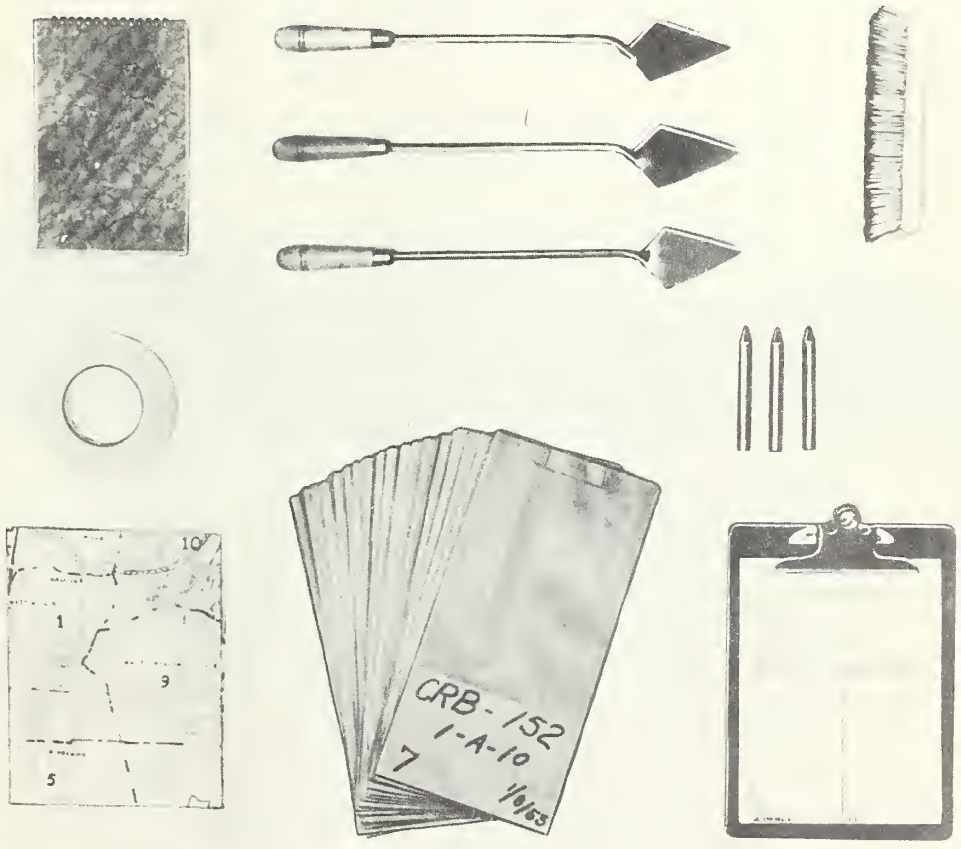


FIGURE 1-SOIL SURVEY EQUIPMENT

FIELD SAMPLING

When a crew is assigned a district or section in which to work, the crew leader contacts the growers concerned. Upon arrival at premises subject to survey, the leader looks over the land, determines the boundaries, size, and shape of the field, and plans how he will survey the property. It has been found advisable to divide a property into several small working units. With such subdivisions, it will be possible to return to a given block for reinspection should infestation be found. It also permits a systematic survey of the field and gives the laboratory a soil sample that is of the proper size for processing. In the initial survey on Long Island the field is divided into working units of 3/4 acre (48 x 72 paces) or 1 acre (48 x 104 paces). (Fig. 2).

The crew leader paces the field to determine its length. Then he divides this length by 72 or 104, depending upon the size of block he expects to work. Adjustments are made in the dimensions of the last block, or blocks, to care for any irregular edges to the field. The initial survey is usually conducted by sampling each block in a grid pattern, collecting soil every 8 paces. This system is commonly called the 8 x 8 block method. The soil samples weigh between 4 and 6 pounds each.

FIGURE 2—SOIL SURVEY OF TYPICAL LONG ISLAND POTATO FIELD

In a field 190 paces long, the leader starts the first man at the edge of the field with bags numbered 1 through 3, each of the first two bags contain soil samples from a section of the row 72 paces long and the third bag from a section 46 paces long. The crew member starts up the edge of the field picking up about a tablespoonful of soil (Figure 4) on a pointing trowel at intervals of 8 paces and putting it in bag Number 1, until he has gone up 72 paces (Fig. 3). He then steps over 8 paces into the field at a right angle and sets bag No. 1 down. He comes back to the first row and resumes operations, using bag No. 2, until he has covered another 72 paces. Again he steps over into the field 8 paces, which brings him in line with bag No. 1, and sets down bag No. 2. He returns to the spot where he left off on the original row and continues with bag No. 3. After 66 paces, with soil samples being placed in bag No. 3, he comes to the end of the field. He again steps over 8 paces along the back edge of the field coming in line with bags Nos. 1 and 2. He starts back toward the road from this point picking up soil every 8 paces until he reaches bag No. 2. Then he steps over 8 paces further into the field and sets down bag No. 3, returns and works bag No. 2 to No. 1, etc. He continues this procedure until he has completed three return trips from the road to the back end of the field. On his last trip back to the road he notes that bag No. 3 is completed when he reaches bag No. 2. He simply folds the top of this bag, which now contains a completed soil sample, carries it under his arm, and continues this procedure until he reaches the road. The leader lays the bags necessary for a tier every 48 paces along the front of the field. Therefore, when the first man completes his tier and reaches the road, he finds that the second man has started his tier just 8 paces over in the field from where he finished. He simply follows along the edge of the field until he comes to the next tier not being worked and begins again.

One other 8 x 8 pace method is used whenever stepping across potato rows in the block method might be injurious to mature or nearly mature potato vines. It is called the strip method and uses exactly the same overall dimensions and trowel dips in each soil sample as in the block method. However, instead of 48 x 72 paces for a sample, the dimensions 8 x 432 paces are used to give approximately 3/4 of an acre. To avoid injury to plants by stepping over potato rows the sampling is done throughout the length of the field.

For reinspection purposes, or when a field or portion of a field is under suspicion or shows symptoms of infestation, it is advisable to divide the field into smaller blocks and to inspect it intensively on a 4 x 4 or 2 x 2 pace pattern. In such cases, the block is reduced to 24 x 36 or 12 x 18 paces for the respective pattern.

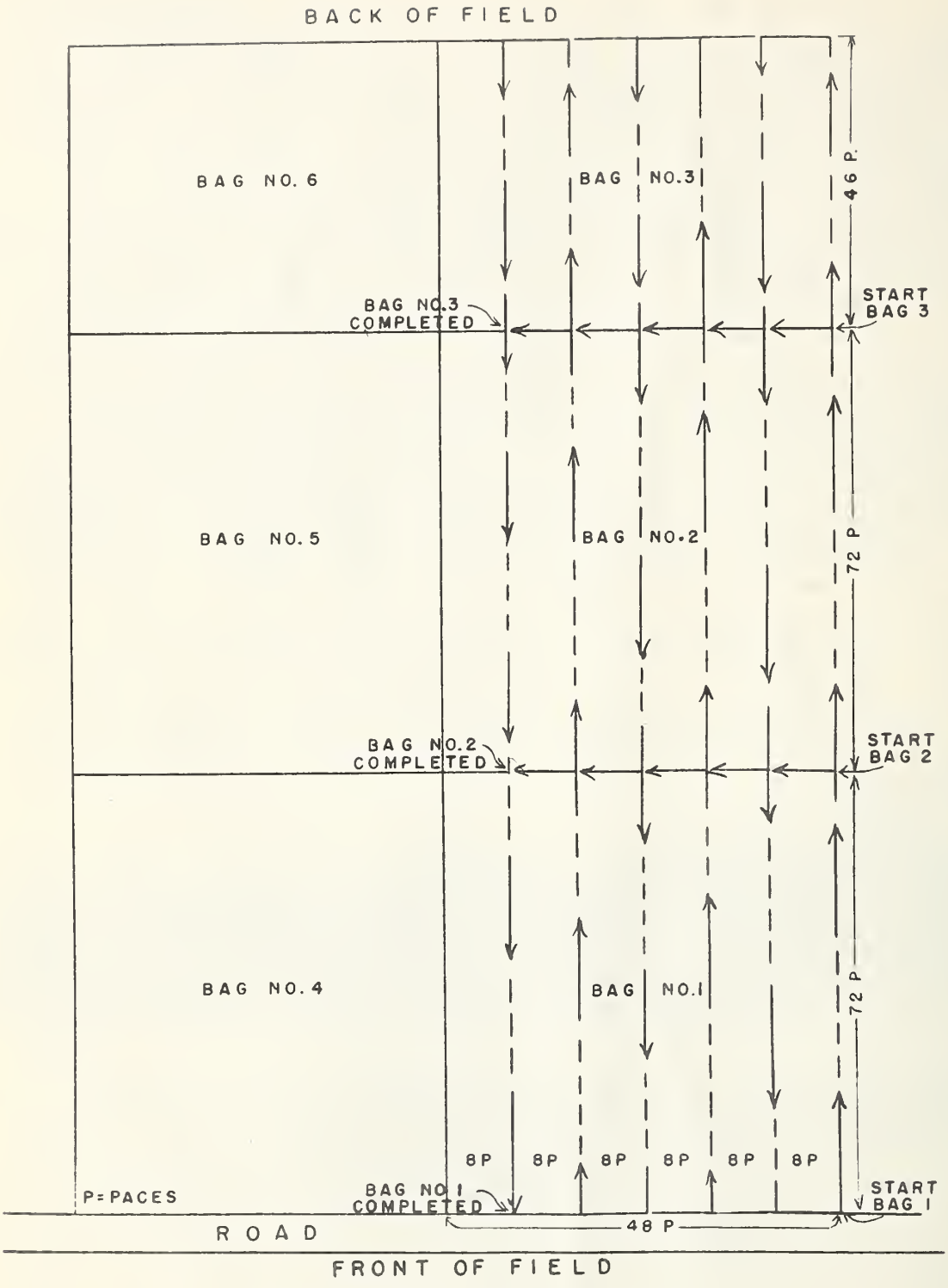


FIGURE 3-FIELD SURVEY METHOD

COLLECTION OF GRADER SAMPLES

Soil is obtained from accumulations under the grader, under the loading belt, in storage bins, or in any location where potatoes are concentrated. Frequently, some farmers place grader debris in burlap bags, baskets, and other containers in the storage cellar for removal to the field and disposal. Other growers merely dump it in piles around the storage cellar. These sites are excellent sources of soil samples. Such debris has a high content of potato vines, sticks, stones, potato skins, and other extraneous offal. Care is taken to include as little of this debris as possible. Dirt from each pile is included in the sample. Where large quantities of soil are available, it is desirable to obtain two or more samples. Each paper bag is filled to within 3 inches of the top, folded and sealed with tape. Every precaution is taken to avoid leakage of soil from the bags. For essential form used in the collection of grader samples see Fig. 8.

PLANT-ROOT EXAMINATION METHOD

A method of examining potato roots for nematodes may be used to advantage under certain conditions. The best time is while the nematodes are in the white or orange stage. On Long Island this is about the last two weeks in June. The cysts are generally in this stage when the potatoes begin to blossom. Fields are looked over carefully, and patches showing plants with weak, spindly stems and stunted tops are selected. Plant root examination should also be made around buildings or where grading debris has been disposed of. The plant is carefully removed from the ground with a pointing trowel. The roots are separated from the soil, but no attempt is made to remove soil that is clinging to individual roots, (Fig. 5), as unnecessary handling causes the nematodes to fall from the roots. With the aid of a hand lens, the root system is examined carefully for female cysts. Specimens collected in this manner are placed in vials containing formalin and referred to the laboratory for determination.

NURSERY AND OTHER SURVEYS

The collection of soil samples from nurseries, greenhouses, cold frames, plant beds, baggage and cargo presents special problems. Obviously, it would be impractical to survey a nursery on an 8 x 8 pattern basis. Therefore, the crew leader should divide the nursery into sections according to the type of stock grown or by following natural boundaries such as roads and walk-ways, and then soil samples should be collected and notations made as to where each originates. This method will apply also to survey of greenhouses. In examining plants being shipped through the mail it may be necessary to wash the roots to determine whether golden nematode cysts are present. Sometimes debris in the bottom of a packing case or bag may be the only material for examination.

LABELING AND RECORDING

One of the most important steps is the proper labeling and recording of the soil samples (Fig. 4). The bag should be labeled in such a manner that all information is visible after the bag has been sealed. The inspector's collection number, which consists of his initials and a number is recorded on the top crease of the bag. The first collection made on the survey is No. 1. Each collection thereafter, regardless of the State or County, is numbered in a series. Thus one inspector's first collection appears as "CRB-1", the second collection as "CRB-2", etc. The name of the farmer, grading house, map designation, or field number is placed on the second crease. The date (month and day only) is recorded in the lower right-hand corner of the bag. The samples obtained on each collection in a given location are also numbered in series beginning with No. 1. On the last sample in a series, following the sample number, the notation "End" is made.

Form GN-2 will be used for field survey. (Fig. 7-A) It is important that this form be filled out in its entirety, in duplicate and that the information requested be stated clearly. In the left-hand column, first line, the date of the survey is recorded. If there is a map designation to the property, it is placed on line 2. Line 3 consists of the crew leader's collection number. Line 4 and 5 denote the number of acres in the property and the number of soil samples collected. On line 6 the "survey pattern" is recorded - whether 8 x 8, 4 x 4 or 2 x 2 pace method. On line 7, a check is put in the box applicable to the type of survey. On line 8, a check is put in the box applicable to the kind of survey.

In the right-hand column, the location of the stored samples is recorded on line 1. On line 2, the operator of the field surveyed is recorded. The operator's mail address is recorded on line 3 and a brief description of the location of the property is given on lines 4 and 5. The location of the field surveyed as to state and county is given, followed by the names of the crew leader and men who assisted him in the survey. No further data is recorded by field survey personnel on the front of this form.

On the reverse side of Form GN-2, a simple diagram of the property inspected is drawn (Fig. 7-B). On this diagram is noted the location of each soil sample collected and the dimensions of the block from which the sample is taken. Enough landmarks should be shown, together with names of roads, telephone pole number, etc., so that the field may be relocated without difficulty. On the lower portion of the form the inspector indicates the type of crop on the field at the time it is inspected and other information, such as whether the crop has been harvested or not. In the lower right-hand corner the leader indicates North on the compass symbol.

Form GN-2a is used for recording information relative to the collection of grader samples (Fig. 8). This report is also made in duplicate, on the first line the state, county, inspector's name and the year are recorded. In column 1 there is placed the collection number; in column 2,

the date of collection; in column 3, the operator's name; in column 4, mailing address of the operator; in column 5, farm storage house or packing house location; in column 6, total acres grown by the farmer (this information may not always be available, but if possible, obtain the nearest approximate figure); in column 7, the approximate acreage of potatoes represented by the sample collected. (For example, if a farmer grows 50 acres of potatoes all stored in one cellar, and he is half through shipping when soil is secured from the 25 acres shipped, the entry in this column would be 25 acres.) In column 8, the number of samples collected at a given barn or warehouse are indicated. Columns 9, 10 and 11 are to be left blank, as well as the last line of the page. These entries are to be made by the laboratory personnel at the time the soil samples are processed. In general, one soil sample is collected for each ten acres of potatoes represented by a pile of soil or debris.

Forms GN-2 and GN-2a, Field Survey Reports, together with narrative of the week's operation, will be submitted in duplicate to the Headquarter's office weekly.

SANITATION

Every reasonable precaution should be taken to prevent the spread of this organism. Vehicles assigned to a survey are not permitted to enter any property. They must remain on paved highways or thoroughfares, and must be kept clean at all times. Trowels must be free of soil-collecting recesses and grooves. A brush is provided for cleaning the inspector's shoes as he leaves fields or storage houses (Fig. 6).

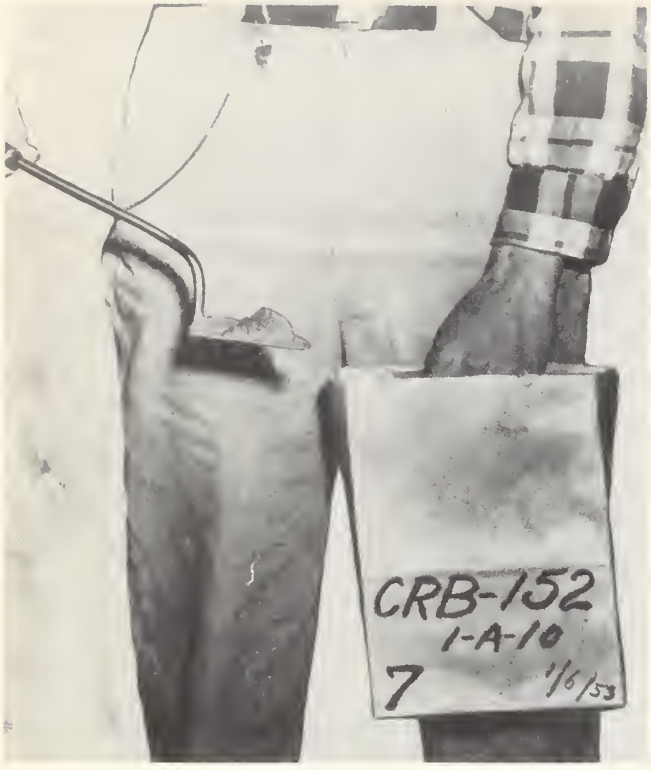


FIGURE 4
SOIL SAMPLE BAG PROPERLY LABELED

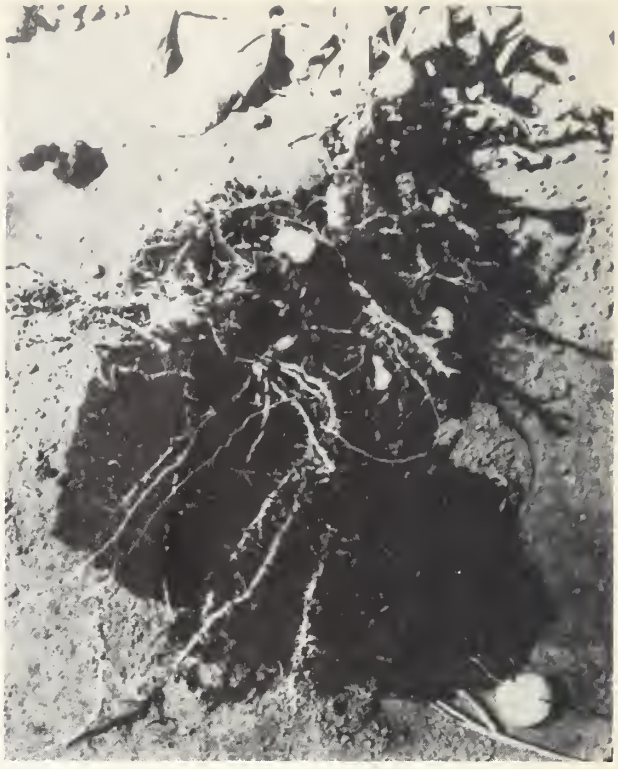


FIGURE 5
ROOTS EXPOSED FOR EXAMINATION

FIGURE 6- SANITARY PRECAUTIONS OF A SOIL SURVEY CREW

FORM GN-2 HICKSVILLE L. I. N.Y.

U.S. DEPARTMENT OF AGRICULTURE A.R.S. PLANT PEST CONTROL BRANCH

GOLDEN NEMATODE SURVEY

DATE 7/18/52 SHELF NO. 31-32 B

MAP DESIGNATION 52-W-7 FARM OPERATOR JOHN DOE

COLLECTION NO. CRB 176 MAIL ADDRESS 100 WEST ST & RURAL AVE.

ACRES SURVEYED 25 FIELD LOCATION N/E COR. WEST ST. & RURAL AVE. RURALTON

NO. SAMPLES COLLECTED 34

SURVEY PATTERN 8 X 8 STATE N.Y. COUNTY NASSAU

☑ INITIAL ☐ CONFIRMATORY ☐ DELIMITING INSPECTORS BENNETT. JONES,

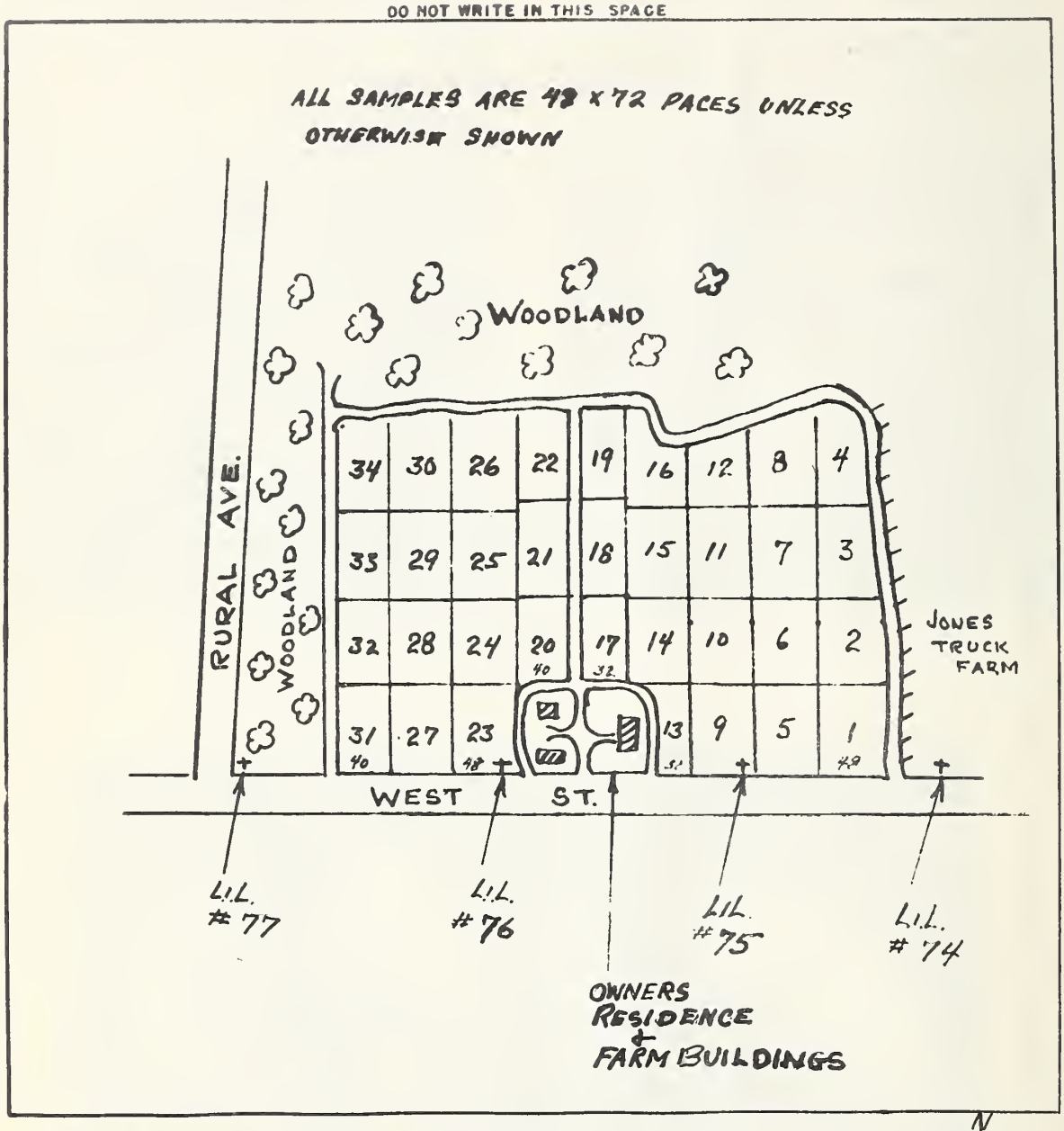
KIND OF SURVEY ☑ POTATO ☐ NURSERY ☐ GREENHOUSE ☐ TOMATO ☐ OTHER

POSITIVE SAMPLE NO.	NO. G.N. CYSTS	IDENTIFICATION NO. OF SLIDES & VIALS FILED	POSITIVE SAMPLE NO.	NO. G.N. CYSTS	IDENTIFICATION NO. OF SLIDES & VIALS FILED
13	1	A 17-20 H. ros.			
14	5	5 CYSTS - VIAL 12			

DETERMINED BY D O'Betz DATE 12/5/52

FIGURE 7A-(FRONT)

DO NOT WRITE IN THIS SPACE

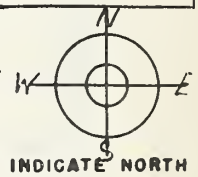


TYPE OF CROP ON PROPERTY & 25 ACRES OF POTATOES

ESTIMATED ACREAGE OF EACH

STATUS OF FIELD AT TIME OF SURVEY HARVESTED

REMARKS



INDICATE NORTH

FIGURE 7B-(BACK),

GN-2a (Revised 4/6/54)

GOLDEN NEMATODE SURVEY
GRADER AND FIELD INSPECTION RECORD

State _____ County _____ Inspector(s) _____ Year 19

Coll. No.	Date	Operator	Address	Location	GRADER		FIELD		Neg.	No. Cysts	Ident. # of Slides & Vials Filed
					Acres	Samples	Acres	Samples			

Determined by _____ Date _____

FIGURE 8-GN-2A

LABORATORY METHODS

INTRODUCTION

This section outlines methods of recovering golden nematode cysts from soil samples. Procedures in operation at the laboratories of the Golden Nematode Control Project are based on the fact that these cysts will float. This is particularly true when the samples have dried. Hence, before being processed they are stored on ventilated racks (Fig. 10). To make determinations in a minimum amount of time and without sacrificing accuracy, there has been developed a two-phase processing cycle, a washing phase and an examination phase. By this means, laboratory personnel may alternate between washing and examining soil samples, and thereby be relieved of the monotony of doing either phase for eight hours each day.

FIGURE 9-INTERIOR OF LABORATORY

The need for infallible determinations has created important procedures aimed at eliminating contamination possibilities. Neat work is required of all personnel including trainees; equipment is thoroughly washed after the processing of each soil sample; shelves and racks bearing samples are brushed and washed before reloading with new samples; and when infested material is found, sieves in use at the time are replaced until they can be thoroughly cleaned and inspected.

FIGURE 10-SOIL RACKS

SPECIAL EQUIPMENT

As in most projects, special problems have required special equipment. Testing sieves currently in use are made by tinsmiths from medium-gage tin. The finished product has the appearance of a 4-inch deep pan with a bottom made of brass screening (Fig. 11).

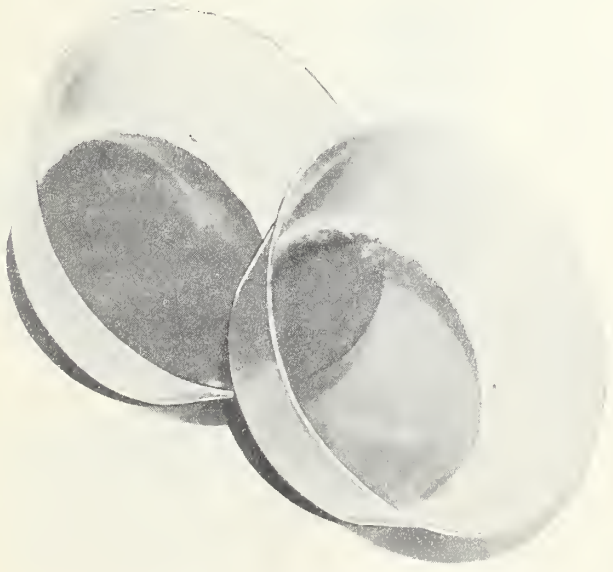


FIGURE 11-TESTING SIEVES

The bottom diameter is 8 inches and the top diameter 10½ inches. For this purpose such a sieve is superior to the factory-made, nesting type of testing sieve, in that it has a greater capacity and freedom of movement and is more rugged.

Where the manual method of washing soil samples is used, special stands have been designed to hold testing sieves in place while material is being poured through them (Fig. 12).

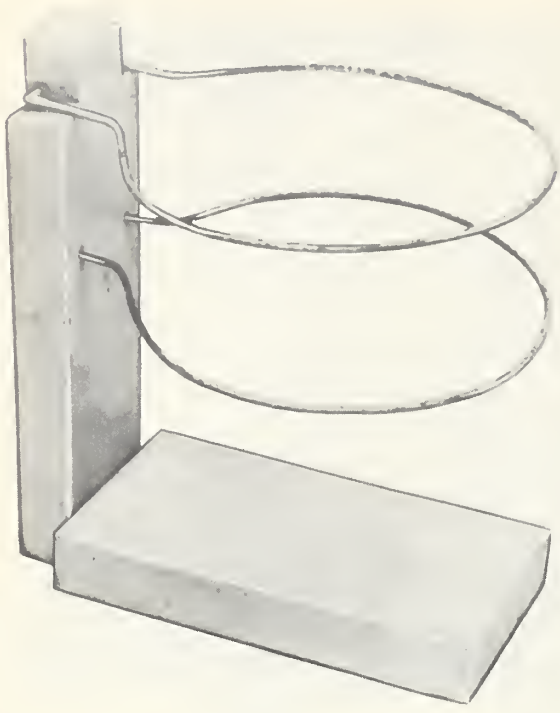


FIGURE 12
STAND HOLDING TESTING SIEVES

These stands are L-shaped, with the base made from a 1-foot section of 2 x 6 plank and the upright a 1-foot piece of 2 x 4.

To the upright section two heavy wire hoops are attached 3 inches apart. The lower hoop, which holds a 60-mesh sieve, is immovable, and the upper hoop, which holds a 20-mesh sieve, is hinged so that it can be moved away to avoid interference with manipulation of the lower screen. (Fig. 13).

Machines are now used for washing soil samples. In less time they do a mo re efficient job with much less fatigue to the operator. They are especially well adapted for setting up on-the-spot temporary laboratories, and thus reduce the need of transporting dangerous soils from one area to another. After several years of use, practical remodeling has brought the soil washing machine to the form illustrated in Figure 14.

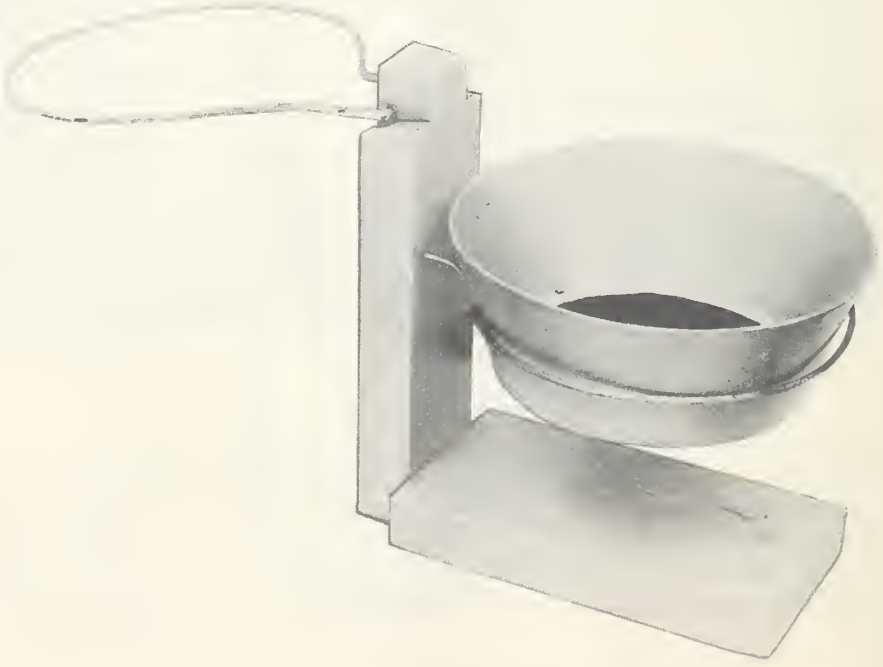


FIGURE 13

POSITION FOR
LOWER SIEVE
MANIPULATION

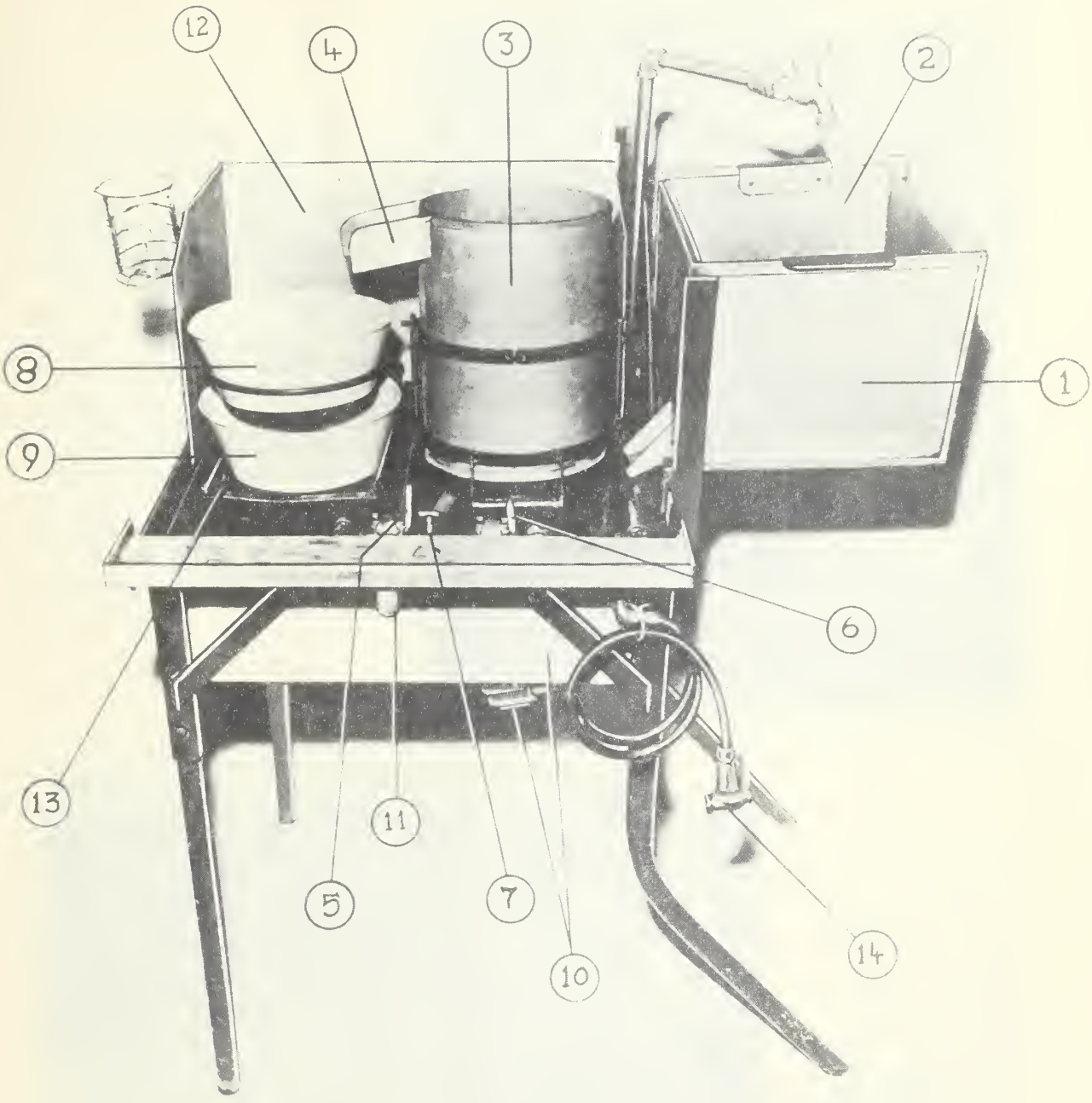


FIGURE 14—SOIL WASHING MACHINE

1 - Sink
2 - Nesting unit
3 - Flotation tank
4 - Spout
5 - Slow overflow, valve 1
6 - Fast overflow, valve 2
7 - Adjusting valve
8 - 20-Mesh sieve
9 - 60-Mesh sieve
10 - Drainage basin and spout
11 - Hose fitting
12 - Splash shield
13 - Sieve receptacle
14 - Hand spray

FIGURE 15-VALVE 1 (CIRCLED) IN OPEN POSITION

WASHING PROCEDURES

Machine Method: Before a sample is introduced into the machine, the slow overflow valve (No. 1) is opened.

This keeps the apertures through which water enters the flotation tank from being contaminated and clogged with soil (Fig. 15).

The identity of every sample must be maintained throughout the procedure (Fig. 16).

It is important that the beaker and the sample bear the same number and that the technician make certain that he is washing the sample into the proper beaker.

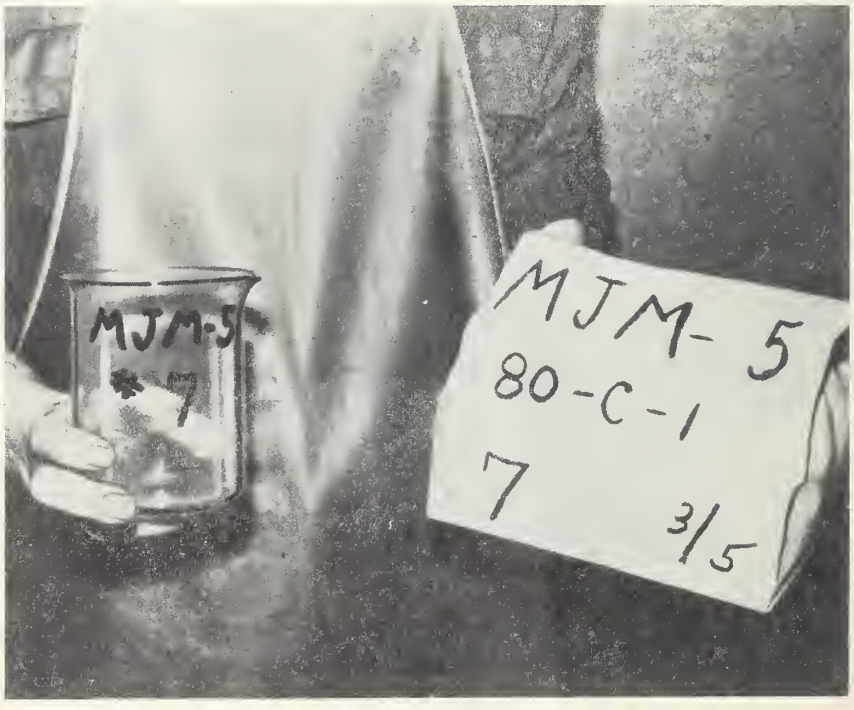


FIGURE 16-MARKINGS ON SAMPLE AND BEAKER

By the time the paper bag containing the soil is opened enough water is in the flotation tank so that the fast overflow valve (No. 2) may be opened and the soil introduced. (Fig. 17)

The fast valve serves to roil and stir the soil. This action is best obtained if the soil is poured in gradually.

FIGURE 17
BOTH VALVES OPEN AND SOIL INTRODUCED

FIGURE 18

OVERFLOWING WITH NO. 2 VALVE CLOSED

The fast valve is closed as soon as material starts to overflow. (Fig. 18).

Overflowing is continued until the water runs fairly clear or until no floating material can be seen on the surface.

Material clinging to the sides of the tank is dislodged and directed through the overflow spout by means of the hand spray (Fig.19).

The spray should be directed laterally along the surface of the water so as not to submerge floating particles.

FIGURE 19
CLEARING SIDES OF TANK

FIGURE 20
SPRAYING MATERIAL
IN TOP SCREEN

Material in the top sieve should be sprayed thoroughly with the hand spray to aid cysts in falling through to the screen below (Fig. 20).

Tests have shown that cysts tend to become trapped in the coarse material in the top screen.

When the flotation tank is free of floating material, it is emptied and cleaned by turning it upside down and opening the fast overflow valve (Fig. 21).

Now the tank is allowed to pivot back to its normal position and the spout is washed outward with the hand spray. Look to see if the tank is washed clean.

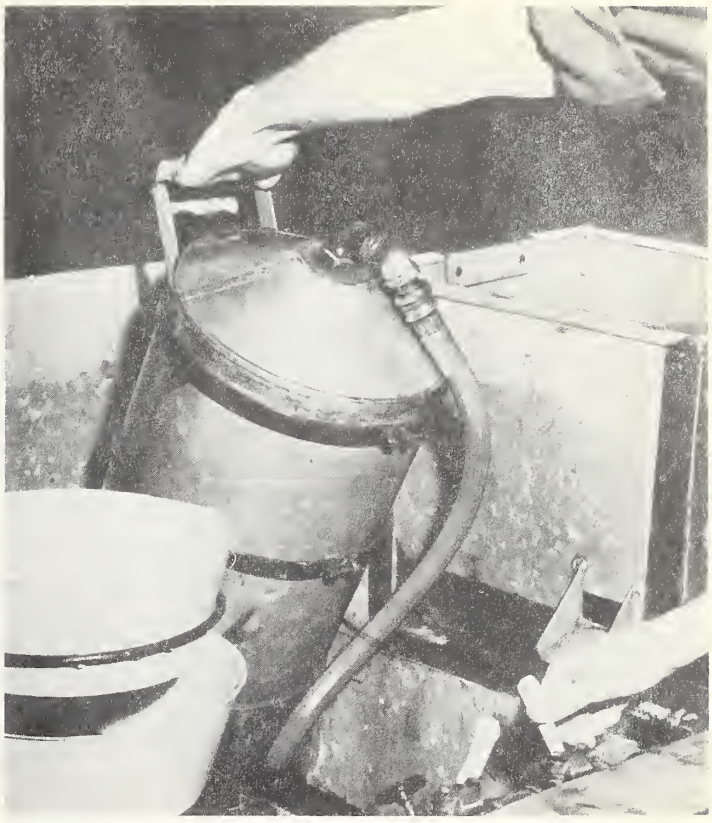


FIGURE 21
CLEANING THE TANK

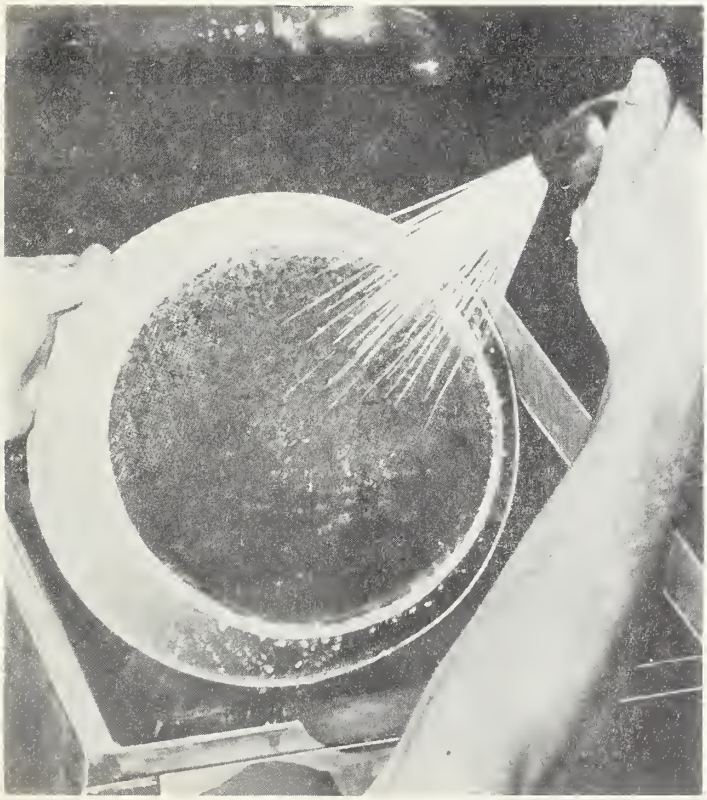


FIGURE 22
CLEANING SCREEN FOR REUSE

Again the material in the top screen is washed using full force on the hand spray.

Then the screen is held over the sink and all surfaces washed, inside and out, with the hand spray (Fig. 22).

FIGURE 23-TRANSFER OF MATERIAL TO BEAKER

Using medium force from the hand spray the material in the bottom sieve is washed and then transferred to a 600 ml. beaker until it is about 3/4 full. (Fig. 23)

This sieve is washed and sprayed in the same manner as the top sieve. After each screen is washed, its holding receptacle should be sprayed clean. After each transfer of material to a beaker, the shelf holding the beaker should also be sprayed clean. The machine is now ready for the next sample.

Manual Method. The purpose of manual washing is to retain all the floating material present in a soil sample for microscopic examination.

The equipment includes three white enameled pails, 2 testing sieves (a 20-mesh sieve for screening out coarse material to be discarded and a 60-mesh sieve which will retain any cysts that may be present), a washstand to support the 20-mesh sieve just above the 60-mesh sieve, a 600-ml. beaker, and a 4-foot length of hose (Fig. 24).

FIGURE 24-EQUIPMENT FOR MANUAL WASHING

FIGURE 25–ROILING SAMPLE

Step 1. The soil sample is placed in one of the enameled pails and roiled with a full force stream of water from the hose (Fig. 25).

All the material that floats is poured into a second pail (Fig.26) and allowed to stand while the first pail is being re-filled by the roiling method.

FIGURE 26

POURING TO SECOND PAIL FOR SETTLING

Again the first pail is decanted, this time into a third pail. If particles of floating material are still clinging to the sides of the pail, it is filled and emptied into the second pail following step 2.

FIGURE 27-POURING THROUGH SIEVES

Step 2. The contents of the second pail, minus soil that has settled to the bottom, are poured through the testing sieves (Fig. 27). This pail is re-filled in order to collect the floating material left after the first pour-off.

Step 3. The same treatment is given the third pail. The alternating process of filling and decanting is continued until it is judged that all floating material has been removed from the soil sample.

The coarse material caught in the 20-mesh sieve is discarded. The material retained in the 60-mesh sieve is washed and transferred to a 600-ml. beaker that has been labeled with the collection number and the soil sample number (Fig. 28).

It is important to remember that soil from different areas varies markedly in the amount of organic matter present. In general, the procedure as described may be taken as the minimum washing necessary. Further washing is left to the good judgment of the individual. A complete washing cycle includes thorough cleaning of all equipment.

FIGURE 28-TRANSFER OF MATERIAL TO BEAKER

EXAMINATION

The purpose of examination is to recover for identification, nematode cysts that may be present in the soil sample. The equipment needed includes a microscope (binocular with 15 x magnification), small 80-mesh sieve (about the size of a muffin tin), Syracuse dishes, scalpel, forceps, needle, medicine droppers with rubber bulb, 600 and 250-ml. beakers, 500-ml. metric bottles, and a lamp.

Technique: Floating material which has been transferred into 600-ml. beakers by the washers is poured into the small 80-mesh sieve. While pouring, the beaker is rotated in such a way as to clear the sides of adhering material (Fig. 29). A scalpel is used for transferring this material into the Syracuse dishes (Fig. 30). The correct amount of material per dish is about ½ teaspoonful. Using the medicine dropper, particles adhering to the scalpel and sieve are rinsed into one of the Syracuse dishes. (Fig. 31). Sufficient water is added to float the material at a level near the brim of each dish. (Fig. 32).

FIG. 29

FIG. 30

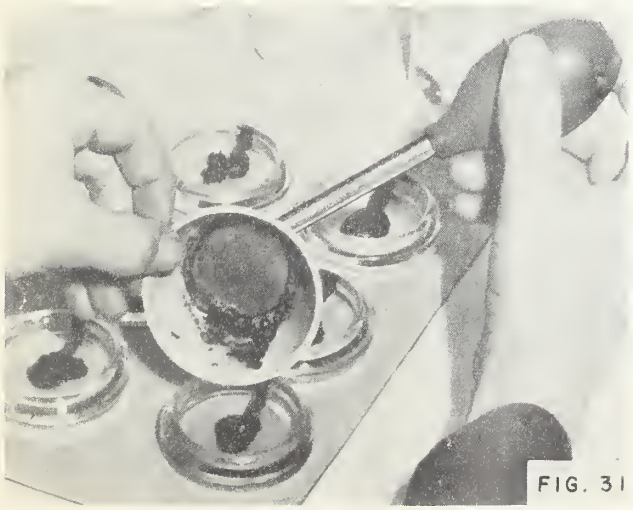


FIG. 31

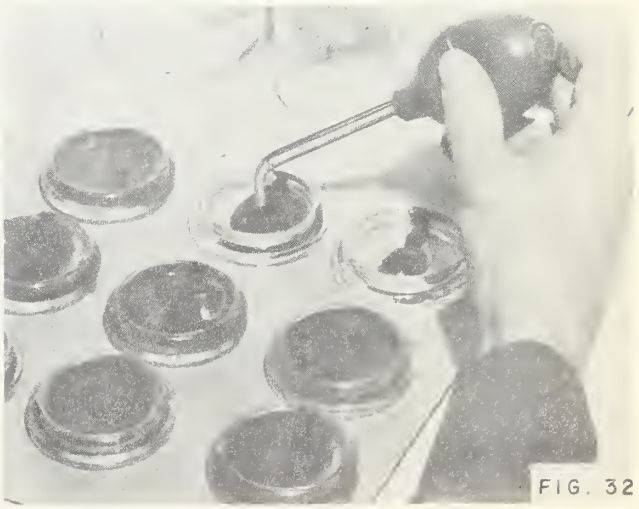


FIG. 32

TECHNIQUES IN EXAMINATION

The Syracuse dishes are marked with a red line in order to identify the area from which examination begins. With the field of view focused on this area, the dish is revolved at a rate that will allow the eye sufficient time for examination of the material before it passes out of view. When the starting position (the red line) reappears, another inner revolution is made to insure examination of portions of the material previously excluded from vision. Then the area in the center of the dish is included using a criss-crossing movement (Fig. 33).

A dish without the red line may be examined by grasping it with the thumb and index finger and moving it in a circle with the field of view following the edge of the dish. When the index finger reaches its original position, a complete circle has been effected. A probing needle should be held in the free hand for use in spreading clumps of material and for closer inspection of cyst-like objects.

Upon completion of the examination, all equipment is thoroughly washed to avoid contaminating the next sample. Suspect cysts are transferred to frosted dishes and identified with collection and sample numbers. All 80-mesh sieves that have contained positive material must be examined under the microscope before they are used for another sample.

FIGURE 33-EXAMINING MATERIAL FOR CYSTS

PREPARATION OF MICROSCOPE SLIDES

Purpose: To identify nematode cysts. The slide is filed for future reference and serves as a record of infestations.

Equipment: Glass slides with one end frosted, No. 1 thin cover glasses, dissecting needles, dissecting scalpel, forceps, slide labels, small brush, ringing stand.

Formulas: Mounting media

Formalin mounts (ring with Zut)
5% formalin, 95 parts
Glycerine, 5 parts

Lacto-phenol mounts (ring with Clearcol)
Phenol, 60 cc.
Lactic acid, 46 cc.
Glycerine, 96 cc.

Sealing compounds

* Thorne's Zut (thin with butyl acetate or acetone)
Obtainable from:

George Gurr	or	Lyman Hunter
136 New Kings Road		Bennett Glass & Paint Co.
London, S.W. 6, England		Salt Lake City, Utah
(under name "Glyceel")		

* Clearcol
Obtainable from:
H. W. Clark
5419 32nd St., N.W.
Washington, D. C.

Formalin mounts--ring with 50-50 mixture of paraffin and vaseline.

Technique: On the frosted end of the slide, record the collection number and number of the sample from which the cyst is recovered. Place a small drop of water on the slide just to the right of center. Then transfer the cyst into the water and cut off the posterior third. This section is the most important and should show the following identifying characteristics: vulva, anus, punctation, and or pattern. It is called the perineal section. The remaining section is then cut in half, with care to leave the neck of the cyst intact. Now remove larvae and eggs from the sections by gently scraping with a needle. If only eggs are present, larvae may be released by applying pressure on the eggs. The presence of larvae on a slide is very important for further identification and for viability determinations.

* These compounds have been used by Branch workers, however, any sealing compounds of a similar nature should be satisfactory.

Arrange the three sections in a row close together with the exterior surface up. Add a drop of the mounting medium and spread over an area about the shape and size of the cover glass. Check the arrangement and position of the sections then apply the cover glass. Remove excess fluid by blotting with a piece of paper towel, but do not allow the cyst contents to be drawn off with the fluid. Seal the edge of the cover glass with a suitable compound such as Zut. For this purpose use a small brush and a ringing stand. The same procedure applies in the case of lacto-phenol mounts which may be sealed with clearcol. Finally, place a gummed label on the left end of the slide (opposite the frosted end) and record information as indicated in Figure 34.

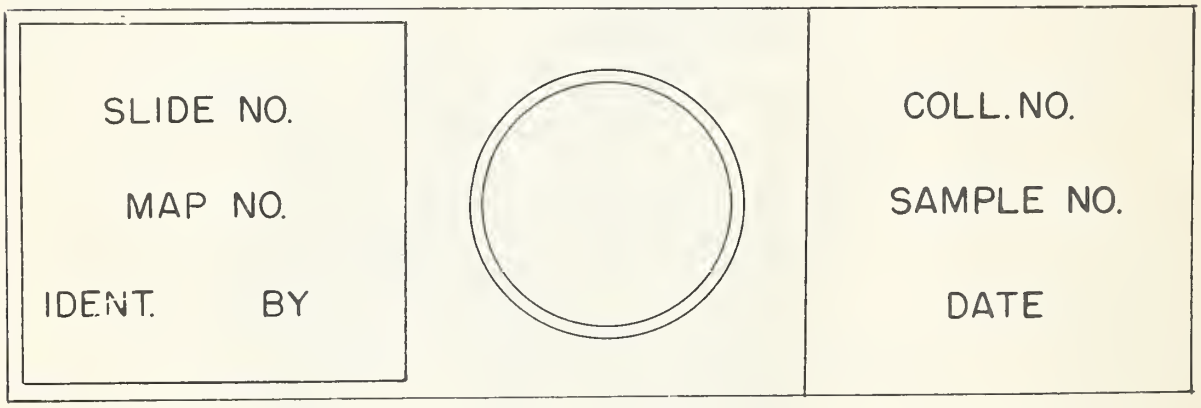


FIGURE 34—A SLIDE PROPERLY LABELED

Slides of cysts from all properties are sent to the Section of Nematology, Horticultural Crops Research Branch, U. S. Dept. of Agriculture at Beltsville, Md. for identification by nematologists. If possible, two slides are made, one for our files and one for Beltsville. All slides sent to Beltsville are recorded on the laboratory form labeled "Record of slides sent to Beltsville". Slides to be mailed are wrapped in cellophane and placed in the wooden holders. Form EQ-449 (Fig.43) is prepared (4 copies) and used for all specimens except those taken from material sent in by Plant Quarantine officials. In the latter case, form EQ-9 is used. The second carbon is dated and kept in our files. All other copies accompany the slide - the original copy is marked "Return to Hicksville", and the last two carbon copies are marked "retain" and are kept by the Section of Nematology for further distribution.

PREPARATION OF VIALS

Purpose: To preserve and make record of cysts occuring in quantity.

Equipment: 5-percent formalin, 2-ml. shell vials, 35-ml. bottles and stoppers, cotton, labels, forceps.

Technique: The shell vial is filled about two-thirds full with 5-percent formalin. Cysts are transferred into the formalin and the vial is stoppered. The number of cysts found in each positive sample is recorded on a slip of paper, together with the collection number, map designation, date, and vial number (Fig. 35).

COLL. NO. MAP NO. DATE VIAL NO.	SAMPLE NO.	NO. CYSTS

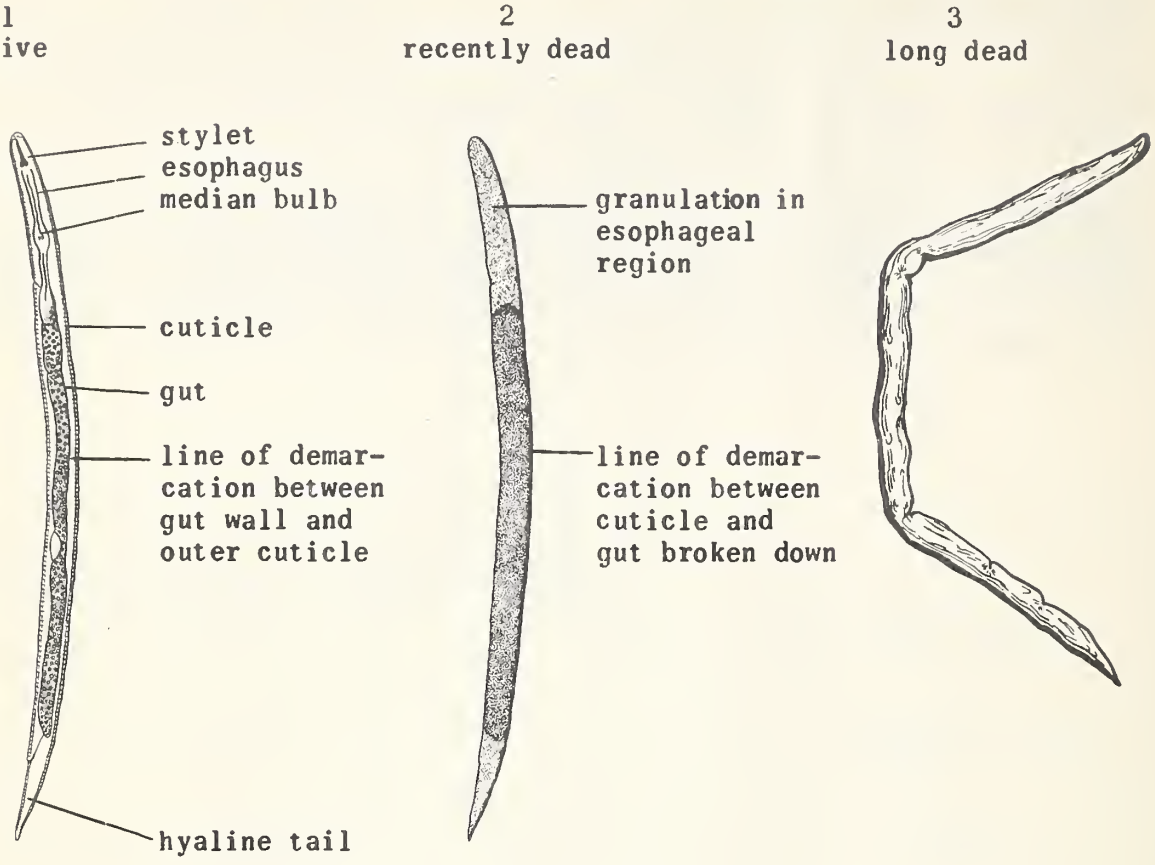
FIGURE 35—INFORMATION TO ACCOMPANY PRESERVED CYSTS

Place the slip of paper in a bottle and add a wad of cotton. Next insert the shell vial containing the preserved specimens. Pack cotton around and above the shell vial for protection against breakage. Cap bottles and number them consecutively. Record these numbers on the laboratory logs and the GN-2 forms.

VIABILITY DETERMINATIONS

Viability of cyst contents must be determined with a great deal of accuracy, since the quarantine action may be dependent upon such determinations. Persons making them must have criteria upon which to judge viability, and procedure must be as simple as possible. The following outline prepared by nematologists of the Section of Nematology, Horticultural Crops Research Branch may be used as a guide.

FIGURE 36-LARVAL CHARACTERISTICS FOR DETERMINING VIABILITY



1. Stylet plainly visible
   Esophageal area-hyaline, clear, structures visible
   Gut full--i.e., black
   Line of demarcation between cuticle and gut wall
   No kinks, may or may not be movement
   Posterior area clear

2. Stylet only faintly visible or not visible
   Esophageal area cloudy, brownish tinge
   Gut either empty or vacuolated--granulation (Perfectly healthy larvae use material in the gut as food if they cannot reach food. As this stored food is used, vacuolation may occur in the mass, and if all the stored food is used, a viable larva may have an empty gut.)
   May or may not be kinks, no movement
   Tail area granular.

3. Kinks in larvae vary in intensity and denote dead larvae.

Viable larvae have turgor, non-viable larvae do not have turgor. Therefore, if a larva is cut transversely with a needle or a knife, the contents of the viable larva will "mushroom" (Fig. 37); the non-viable larva will show little visible reaction to cutting (Fig. 38).

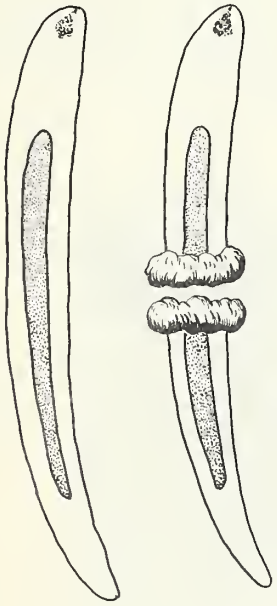


FIGURE 37-VIABLE

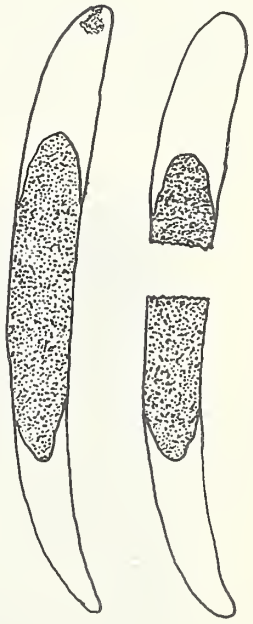


FIGURE 38-NONVIABLE

Movement can often be detected by placing a bamboo sliver across the larva at a nerve center just posterior to the median bulb, and gently massaging with a rolling motion.

GUIDE FOR TRAINING NEW PERSONNEL

Equipment: Cyst-infested soil, Syracuse dishes, needles, forceps, microscope.

Note: Neatness must be stressed in order to avoid bad habits which might result in contamination.

Procedure: Step 1: Important external identification characteristics are explained to the trainees. They are:

Color...is the characteristic that first catches the eye and leads to further examination of a suspect object. The color range is from yellow to dark bronze.

Shape...Typically, the cyst of the golden nematode is spherical.

Size....Actual measurements are not taken but a cyst is compared with other objects that will at first confuse the prospective inspector. Microcysts are usually smaller than golden nematode cysts. Some of the seeds and seed pods are much larger in comparison (Fig. 39).

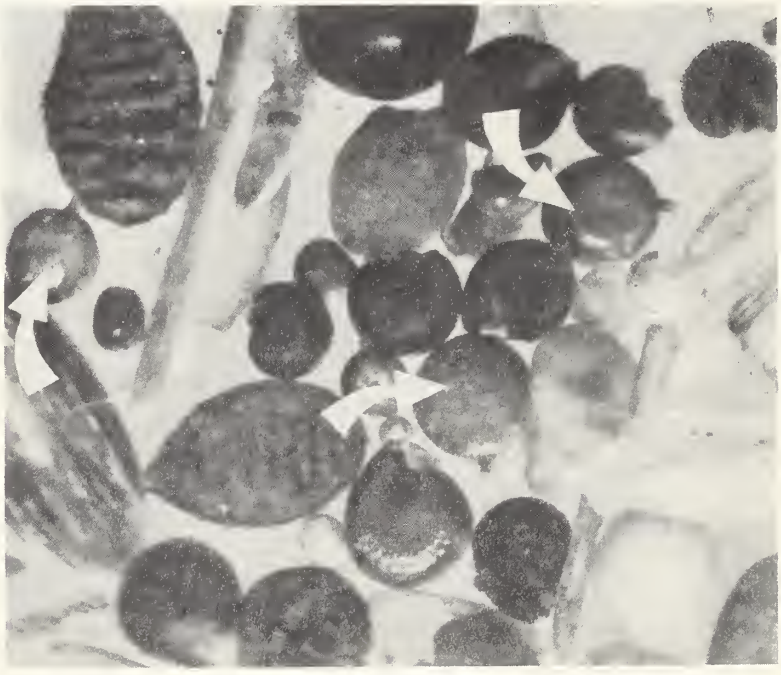


FIGURE 39
MICROSCOPIC VIEW OF CYSTS IN FLOTSAM
(← INDICATES GOLDEN NEMATODE CYST)

Texture of cyst wall...Suspect material will show different reactions to pressure from a needle. This technique is called the "feel of the needle", and it is acquired only through handling of the material. Microcysts, for example, will usually break like an egg shell when needle pressure is applied. Many seeds will feel hard like stone. Seed pods are usually tougher than the cyst wall of a nematode. The latter will yield to pressure without breaking or tearing.

The prospective inspector then becomes acquainted with cysts by picking out specimens from infested material. The objects he picks out are checked by an authority who demonstrates differences between the golden nematode cyst, microcyst, seed, seed pods, and H. weissi (a nematode commonly found). The trainee breaks and examines contents of some cysts.

Step 2: As soon as the trainee is able to identify golden nematode cysts from other forms, three Syracuse dishes are set up using examined material that has been discarded by regular laboratory personnel. About six golden nematode cysts, representing the different ranges in color, shape, and size encountered in routine inspections, are placed in the dishes, which are numbered 1, 2, and 3. The trainee finds and transfers golden nematode cysts to a drop of water in a frosted dish. Specimens that he has identified as golden nematode cysts are then checked. As soon as he consistently recovers golden nematode cysts only, it is evident that he recognizes cysts of this species when he sees them.

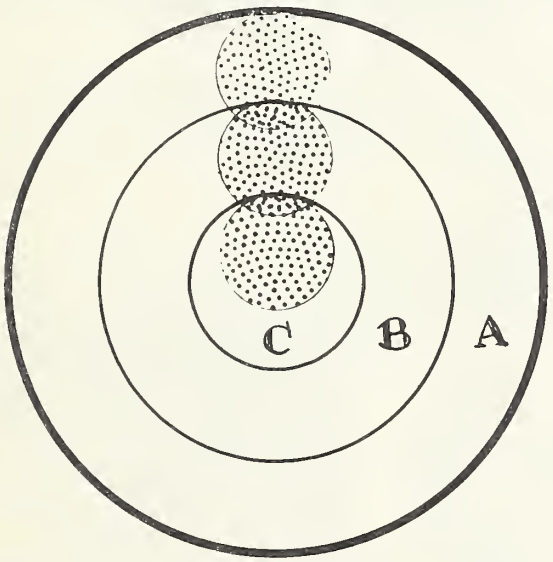


FIGURE 40-AREAS OF A SYRACUSE DISH (DIAGRAMMATIC)

Step 3: It should be made certain that the trainee is observing the entire area of each dish. The size of a microscope field divides the area to be examined into three sections, A, B, and C. The shaded circles in Figure 40 represent the area covered by a single microscope field. With each field of view as a starting point, areas A, B, and C are observed as the dish is turned in a complete circle.

Figure 41 shows a typical test using the three dishes and six golden nematode cysts. Black dots represent golden nematode cysts. By placing cysts in definite areas of each dish and then asking the trainee to find them, the instructor is able to discern those areas which are being overlooked. For example, if the observer consistantly locates the cysts in dishes 1 and 2 (Fig. 41), but misses those in dish 3, it is evident that he is not moving the dish to include inspection of material in the center and corrective measures can be taken. Likewise, dish 1 serves as a means of checking section A, and dish 2, section B.

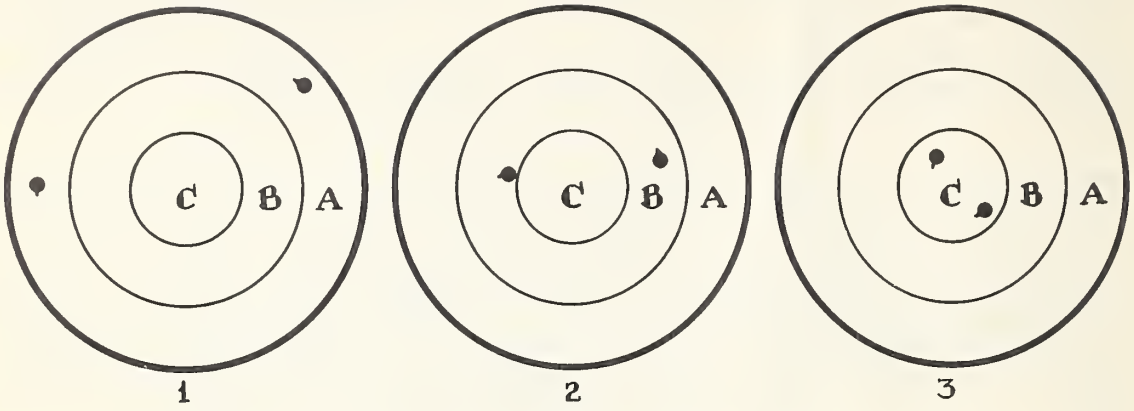


FIGURE 41-TEST FOR TRAINING NEW INSPECTORS (DIAGRAMMATIC)

Step 4: When step 3 is completed in a satisfactory manner and in a reasonable length of time, it is well to increase the knowledge and interest of the trainee through photographs, literature, and slides.

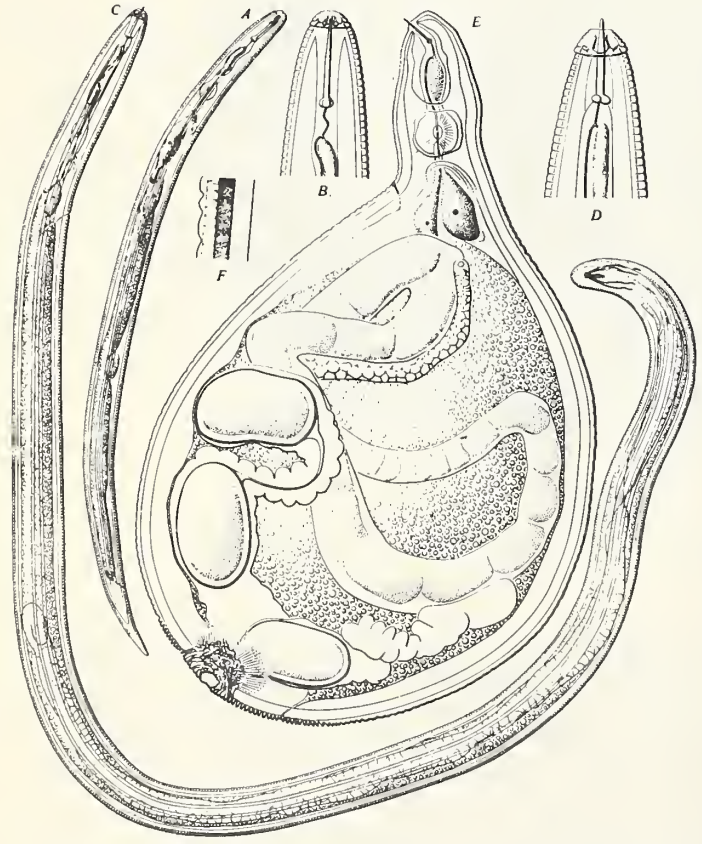


Fig. 42. The golden nematode of potatoes, Heterodera rostochiensis Wollenweber, 1923: A, Infective larva, hatched from egg; B, head of infective larva; C, adult male; D, head of adult male; E, young adult female; F, section of cyst wall. A, C, and E, X 250. B, D, and F, X 580. (From Chitwood, U.S.D.A. Circular 875).

FIGURE 42-THE GOLDEN NEMATODE OF POTATOES

Distinguishing Characteristics Used in Identification of Cysts

Vulva.....rounded, not greatly protruding, but about on a level with the cyst wall.
Anus.....distinct, but much smaller than the vulva.
Cyst wall.....with open, runic pattern, and with punctations in regular horizontal rows.
Larvae.....with rather weak knobs.
Egg.....with plain shell, not punctate.
Larvae and eggs, when present, well protected within the cyst wall.

LABORATORY REPORTS

Original copies of GN-2 forms (Fig. 7-A, page 11) prepared by field crew leaders reach the laboratory through the headquarters' office. These forms concern the laboratory in the following manner:

In case no golden nematode cysts are found, the word "Negative" is stamped on the face of each form and it is signed and dated by the laboratory supervisor on the line marked "Determined by" at the bottom of the page.

If golden nematode cysts are found, each positive sample is recorded by inserting in spaces provided, the sample number, the number of cysts found, and the identification number of slides and vials filed (Fig. 7-A, page 11).

Four copies of EQ-449 forms (Fig. 43, page 36) are prepared for each slide or vial to be sent to the Section of Nematology of the U. S. Dept. of Agriculture. Information called for on these forms is taken from the GN-2 form. One carbon is retained in a pending file and dated; the other forms are submitted with the specimen.

GN-2a forms (Fig. 8, page 13) are completed at the laboratory by filling in the last three columns on the right-hand side, and signed and dated by the laboratory supervisor.

A laboratory log should be maintained as a record for each sample processed (Fig. 44, page 36).

Completed forms GN-2 and GN-2a and a narrative of laboratory activity prepared on form GN-8 will be submitted to the headquarters' office weekly.

Form EQ-449
Revised July 1, 1936

U. S. DEPARTMENT OF AGRICULTURE
BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE

SPECIMENS FOR DETERMINATION

Collection No. JFS 35

Owner JOHN DOE

Address 100 W. MAIN ST. Hicksville N.Y.

County NASSAU

Location N/E COR. W. MAIN ST & 1ST AVE.

Location in grove or field

SAMPLE LOT # 15

Host plant POTATOES

Collector J. F. SPEARS

Date of collection 9/18/52

Other information: SLIDE # B1-20-17
A-17-20 NVHR DOB

Referred for identification to

DR. G. STEINER NEMATOLOGIST

Referred by O.O. BETZ

Determined as H. ROSTOCHIENSIS

Determined by G. S. COBB

Notes: DIV. NEMOTOLOGY

(Send three copies of this form with specimens)

FIGURE 43—FORM EQ-449

LABORATORY LOG

DATE 1/5/53 PAGE 1

COLLECTION NUMBER	BAG NO.	EXAM. BY	NO. CYSTS	SLIDES VIALS DETERMINATION
VAL 75	1	JJ.		
	2	WAA		
	3	JF.		
	4	JJ.	1	B-2-26-18 H. ROST.
	5	JR.	10	10 CYSTS - VIAL 3
	6	WAA		
	7	JJ		
	8	JF.		
	9	JJ		
	10	JR		

FIGURE 44—LABORATORY LOG

NOTES ON TEMPORARY SUB-LABORATORIES

To State, Federal and foreign officials who may be asked to furnish space and accommodations for temporary laboratories, and to supervisors and officials who may be called upon to arrange for laboratory facilities, the following notes will be helpful in selecting a suitable laboratory site.

1. Samples collected weigh from 4 to 6 pounds each.

2. The number of samples processed at a location will vary from several hundred to over 10,000 with an average of about 2,000.

3. At least 4 gallons of water are needed per sample.

4. At most locations it is necessary to dig a pit for the disposal of washed soil, the drainage of water used, and as a precaution against spreading any soil pests. The average pit is 5' x 7' and 6 feet deep.

5. The washing site should be located near the pit. Galvanized pipe (3 to 4 inch down spout) is the usual means of conducting machine washings into the pits.

6. It is preferable to have a water connection within 50 feet of the washing site.

7. One electrical outlet is essential.

8. A place is needed where used paper bags may be burned.

9. It is well to know in advance whether a table and chairs can be furnished at the laboratory site.

10. Ordinary house water pressure is sufficient. However, such pressure may not be maintained on a small water main that has an unusual load requirement.

TECHNIQUES PERTAINING TO FREE LIVING NEMATODES*

SOIL WASHING TECHNIQUES

Equipment:

2 - tin or enamel pans 10 or 12 inches in diameter.
1 - tin or enamel pan 6 inches in diameter.
3 - brass cloth screens 6 inches in diameter of approximately 20, 50 and 150 meshes per inch.
2 - similar screen rims, but without brass cloth. These fit together to hold fine mesh silk used as the last screen.
1 - piece finest mesh bolting silk about 8 inches square. (If 200- or 300-mesh brass cloth screens are available, they should be used in place of the silk cloth.)
3 - beakers, about 250 cc.
3 - beakers, about 150 cc.
3 - beakers, about 50 cc.
1 - sink equipped with water tap about 15 inches above sink.

- - -

Instructions:

1. Place soil sample, 1 or 2 pounds, in one of the pans and cover well with water. Mix thoroughly until all clods are broken. Allow to settle 10 seconds (Fig. 45).

2. Pour muddy solution through 20-mesh screen into second pan, leaving heavy soil particles behind. (Fig. 46) This heavy portion may immediately be discarded.

3. Rinse residues caught on screen by setting it into the pan of muddy water and lifting it up and down two or three times. This also may now be discarded since all nematodes had doubtless gone through it (Fig. 47).

4. Pour the muddy residues through the 50-mesh screen, allowing the portion of heavy material which has settled to remain behind and be discarded.

5. Rinse the residues in the screen by dipping into the pan of muddy water as outlined in step 3.

6. Place the screen on edge in the 6 inch pan, hold it under a small stream from the water tap and wash the residues into the pan, turning the screen so that all portions are washed. The stream should be aimed on the back of the screen (Fig. 48).

* These techniques were furnished through the courtesy of Gerald Thorne, Senior Nematologist, Horticultural Crops Research Branch, Nematology Section, Salt Lake City, Utah.

7. Pour the residues through the same screen again. This should remove most of the fine soil particles, leaving the residues clean upon the screen. Always repeat the operation until all cloudiness disappears.

8. Wash the residues into the 6-inch pan again under the water tap. This should be done with a minimum of water, just about enough to fill one of the 250 cc. beakers.

9. Hold the pan of residues still for about 15 seconds and then carefully pour into the beaker, leaving heavy portions behind to be discarded (Fig. 49). Repeat the operation two or more times if excess sediment still remains.

10. Repeat process as outlined, using the 150-mesh screen, and place the final cleared residues in another beaker.

11. Wet the piece of bolting silk and stretch it over the bottom of one of the open screen rings, then place the second ring over it, forming a silken screen held between the two rings.

12. Hold this silk screen at an angle of about 25 degrees and pour the final muddy residues on to it in a small stream. Hold the silk tight from below with two fingers of the hand holding the screen so that it forms a "drum" which will vibrate as the stream falls on it, otherwise the screen may quickly clog and prevent the muddy residues from going through.

13. Rinse the silk screen residues into the pan and handle as outlined above. Because of their extremely fine nature, a second time through will almost always be necessary to get them clear.

- - -

If correctly followed, this process will give three beakers of fairly clear material which may now be examined under the binocular.

Residues from the 50 mesh screen have been settling for several minutes and all nematodes are on the bottom of the beaker. Now pour off all excess water, leaving only about half an inch in the bottom. Place a small portion of this in a glass dish under the binocular and examine for nematodes. Those caught on this screen will usually be large specimens, 2.0 mm. long or over.

When the course residues have been examined, pour excess water off the 150-mesh residues and examine for nemas. This will contain all except small larvae of the various species.

By the time these are examined, the silk screenings can have the excess water poured off and the residues examined.

- - -

WASHING PROCEDURES
FOR
FREE LIVING NEMATODES

FIG. 45

FIG. 46

FIG. 47

FIG. 48

FIG. 49

This process consists of leaving heavy particles behind in the pans and discarding them, while the extremely fine particles are finally passed through the silk and discarded. Only the nematodes and small pieces of organic matter are retained on the screens and these should always be rinsed until clear to facilitate examination under the binocular.

If possible, practice with a sandy soil which will wash very easily. The heavy clays are much more difficult and a little previous experience will aid in handling them when they are reached. Frequently it will be necessary to rinse screen residues from clay soil three to five times before they come through clean for examination.

By pouring off excess water from the beakers, much work can be avoided, but if the small portion remaining is poured into one of the smaller beakers and allowed to settle a few minutes, a second pouring can be made which will further reduce the quantity, and even a third pouring is frequently used.

If considerable heavy material remains in the residues, pour them into a pan, allow to settle for a few seconds and then pour back into the beakers, leaving the heavier portions behind. This process may be repeated if necessary.

At first, always examine residues from the beakers before discarding. With practice, the operation can be reduced to a comparatively short time.

PICKING OUT THE SPECIMENS

For this purpose, nothing is better than a bamboo splinter sharpened under the binocular to an extremely fine slender point. This may be mounted in the usual laboratory needle holder and is far superior to any metal needles because it can be made smaller and, being slightly rough, the nematodes can be more easily picked from the water.

KILLING AND FIXING

Pick the nematodes into a hollow ground slide after which they should be relaxed by holding over a small flame. Do not over-heat and cook them.

After killing, they should be placed in a good fixative. The common formol, alcohol, acetic acid mixture gives excellent results. It is made as follows: Fix for 24 to 48 hours.

40 cc. - water
20 cc. - 95% alcohol
6 cc. - formalin
1 cc. - acetic acid

From the fixative, they may be removed to 2% glycerin in 30% alcohol and allowed to evaporate slowly. For this purpose the writer prefers small chambers in which tubes of calcium chloride are placed along with the nemas in glycerin solution, about 1 gram per cc. of solution. Place corks in the tubes from which a small section has been cut. This will allow the calcium to absorb the water very slowly, especially near the end of the process, leaving the nemas in almost pure glycerin.

Place the nemas in a large desiccator after making sure that they are completely evaporated and then mounted in glycerin which is also kept in a desiccator. Mount on microscope slides, using 3 pieces of glass rod, just slightly smaller than the nemas, as supports.

Ring with "ZUT", "GLYCEEL" or similar slide-ringing compound.

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DIRECTIONS FOR COLLECTING AND SHIPPING PLANT MATERIAL AND SOIL TO BE EXAMINED FOR NEMATODE INFESTATIONS

Nematodes are, at best, difficult subjects with which to work and it is essential that all material submitted for examination and identification be given the best of care before arriving in the laboratory. Material received in poor condition entails needless work and usually gives unsatisfactory results. The following procedures are suggested to insure the arrival of specimens in the best possible condition.

1. Prevent drying and ship fresh.

    a. Stems and leaves should immediately be packed in moist cotton, paper or moss and wrapped in waxed paper.

    b. Roots should be carefully lifted, not pulled, together with a pound or two of the adhering soil and wrapped in waxed paper. If the soil is dry, moisten before wrapping.

    c. Collect ample material, especially of new or rare forms. Frequently information is limited by meagre collections.

    d. Give supporting data: subject, locality data, collector, symptoms, extent of damage, distribution, etc.

2. Preserve immediately in case fresh shipment is impractical.

    a. An excellent solution is made by adding one part commercial formaldehyde to seven parts of water. Used hot, (but not boiling) it relaxes the nematodes and prevents distortion.

    b. Use other standard fixatives, preferably hot.

REFERENCES

On Nematodes in General:

Chitwood, B. G., and Chitwood, M. B., Nematology Section 1, Anatomy. B. G. Chitwood, 1950.

Filipjev, I. N., and Stekhoven, J. H. Schuurmans, Jr., A Manual of Agricultural Helminthology. E. J. Brill, Leiden, Holland, 1941.

Goodey, T., Soil and Fresh Water Nematodes. Methuen and Co., Ltd., London, 1951.

Hyman, Libby Henrietta. Invertebrates. Vol. 3. McGraw-Hill Book Co., 1951.

Special Groups of Nematodes:

Franklin, Mary T., The Cyst Forming Species of Heterodera. Wilding and Son, Ltd., Shrewsbury, England, 1951.

Steiner, G., Taylor, A. L., and Cobb, Grace S. Cyst-Forming Plant Parasitic Nematodes and Their Spread in Commerce. Pro. Helm. Soc. Wash. 18, (1) pp 13-18 January, 1951.

Steiner, G. Plant Nematodes the Grower Should Know. Soil Sci. Soc. Fla. Proc. 4(B): 72-117. 1949.

Keys on Nematodes:

Allen, M. W., Taxonomic Status of the Bud and Leaf Nematodes Related to Aphelenchoides Fragariae (Retzema Bos. 1891). Proc. Helm. Soc. Wash. 19, (2) July, 1952.

Raski, Dewey J., On The Morphology of Criconemoides, Taylor, 1936, With Descriptions of Six New Species. Proc. Helm. Soc. Wash. 19, (2) July, 1952.

Thorne, Gerald, On the Classification of the Tylenchida, New Order (Nematoda, Phasmidia). Proc. Helm. Soc. Wash. 16 (2) July, 1949.

Thorne, Gerald, A Monograph of the Nematodes of the Superfamily Dorylaimoidea. Capita Zoologica. Vol. 8, Part 5, The Hague, 1939.

Thorne, Gerald and Swanger, Helen Heinly. A Monograph of the Nematode Genera Dorylaimus, Dujardin, Aporcelaimus, N. G., Dorylaimoides, N.G. and Pungentus, N.G. Capita Zoologica Vol.6, Part 4 The Hague, 1936.

On Heterodera rostochiensis (Woll.):

Chitwood, B. G., The Golden Nematode of Potatoes. U. S. Dept. Agr. Cir. 875. 1951.

Grainger, John, The Golden Eelworm. Research Bulletin 10 of the West of Scotland Agricultural College, Auchincruive, Ayr. April, 1951.

Lear, Bert, Mai, W. F., Feldmesser, J., and Spruyt, F. J. Soil Fumigation Experiments on Long Island, New York, To Control Golden Nematode of Potatoes. Phytopath. 42, 193-196. 1952.

Lear, Bert, Use of Methyl Bromide and Other Volatile Chemicals for Soil Fumigation. Cornell Extension Memoir 303, 1951.

Mai, W. F. and Lear, Bert, The Golden Nematode. Cornell Extension Bull. 870.

Mai, W. F. and Lownsbery, B. F. Crop Rotation In Relation To The Golden Nematode Population of the Soil. Phytopath. Vol. 42, No. 7, 345-347, July, 1952.

Mai, W. F., Spruyt, F. J. Lear, Bert, and Feldmesser, J. Yields of Green Mountain and Cobbler Potato Varieties Grown on Golden Nematode Infested Soil. Amer. Potato Jour. 28: 617-625. 1951.

Mai, W. F., and Spears, J. F. Golden Nematode Situation in the United States. Amer. Potato Jour. 1954 in press.

Oostenbrink, M. Het. Aardappelaaltje, Versl. en Med., Plant Dienst. (Holland) 115. 1950.

Spears, J. F. Chemical Control of the Golden Nematode. Agricultural Chemicals Vol. 8, No. 12: 52-54. 1953.

Spears, J. F. The Golden Nematode of Potatoes and Tomatoes - How To Prevent Its Spread. U. S. Dept. of Agriculture Leaflet No. 361.

Taylor, A. L. Chemical Treatment of the Soil for Nematode Control. Advances in Agronomy. Vol. 8. Academic Press, Inc., N. Y. 1951.

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